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TITLE: Application of Physiologically Based Pharmacokinetic Modeling in Understanding Bosutinib Drug-Drug Interactions: Importance of Intestinal P-Glycoprotein

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RUNNING TITLE: Bosutinib PBPK Modeling with Intestinal P-gp Kinetics

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ABBREVIATIONS:

AUC, area under the plasma concentration-time curve; AUCR, AUC ratio; C_{max}, maximum plasma concentration; $C_{max}R$, C_{max} ratio; CL_{int}, intrinsic clearance; DDI, drug-drug interaction; F_a, fraction of the dose absorbed from gastrointestinal tract; F_g, fraction of the dose that escapes intestinal first-pass metabolism; F_h, fraction of the dose that escapes hepatic first-pass metabolism; f_m, fraction of the dose metabolized by an enzyme; f_u, unbound fraction; PBPK, physiologically-based pharmacokinetics; P-gp, P-glycoprotein; PK, pharmacokinetic; SF, scaling factor; t_{max}, time to reach maximum plasma concentration; V_{ss}, volume of distribution at steady-state

ABSTRACT

Bosutinib is an orally available Src/Abl tyrosine kinase inhibitor indicated for the treatment of patients with Ph+ chronic myelogenous leukemia at a clinically recommended dose of 500 mg once daily. Clinical results indicated that increases in bosutinib oral exposures were supra-proportional at the lower doses (50 to 200 mg) and approximately dose-proportional at the higher doses (200 to 600 mg). Bosutinib is a substrate of CYP3A4 and P-glycoprotein and exhibits pH-dependent solubility with moderate intestinal permeability. These findings led us to investigate the factors influencing the underlying pharmacokinetic mechanisms of bosutinib with physiologically-based pharmacokinetic (PBPK) models. Our primary objectives were to: 1) refine the previously developed bosutinib PBPK model based on the latest oral bioavailability data and 2) verify the refined PBPK model with P-glycoprotein kinetics based on the bosutinib drug-drug interaction (DDI) results with ketoconazole and rifampin. Additionally, the verified PBPK model was applied to predict bosutinib DDIs with dual CYP3A/P-glycoprotein inhibitors. The results indicated that 1) the refined PBPK model adequately described the observed plasma concentration-time profiles of bosutinib and 2) the verified PBPK model reasonably predicted the effects of ketoconazole and rifampin on bosutinib exposures by accounting for intestinal P-gp inhibition/induction. These results suggested that bosutinib DDI mechanism could involve not only CYP3A4-mediated metabolism but also P-glycoprotein-mediated efflux on absorption. In summary, P-glycoprotein kinetics could constitute a critical element in the PBPK models to understand the pharmacokinetic mechanism of dual CYP3A/P-glycoprotein substrates such as bosutinib exhibiting nonlinear pharmacokinetics due largely to a saturation of intestinal P-glycoprotein-mediated efflux.

INTRODUCTION

For predicting and understanding pharmacokinetics, drug-drug interactions (DDIs), drug-disease interactions and pediatric/geriatric therapies of new molecular entities (NMEs), mechanistic modeling and simulation approaches are increasingly being applied to all phases of drug discovery and development as well as regulatory decisions on labeling languages (Rowland et al., 2011; Huang and Rowland, 2012; Wagner et al., 2015; Wagner et al., 2016; Shebley et al., 2018). Among the modeling and simulation approaches, a physiologically-based pharmacokinetic (PBPK) model is a powerful tool to quantitatively predict DDIs based on drug-dependent physicochemical and pharmacokinetic parameters along with drug-independent physiological systems parameters (Lave et al., 2007; Nestorov, 2007; Rowland et al., 2011; Jones and Rowland-Yeo, 2013; Jones et al., 2015). Recently, the US Food and Drug Administration (FDA), the European Medicines Agency (EMA) and the Japanese Pharmaceuticals and Medical Devices Agency (PMDA) have issued DDI guidances, which highlight the use of integrated mechanistic approaches including PBPK models (CHMP, 2012; PMDA, 2014; CDER, 2017a; CDER, 2017b). Accordingly, there has been a growing emphasis in developing PBPK models to assess potential DDI risks of NMEs in a drug discovery and development setting.

Bosutinib (Bosulif®), an orally available Src/Abl tyrosine kinase inhibitor, has been approved globally for the treatment of adult patients with chronic, accelerated, or blast phase Ph+ chronic myelogenous leukemia with resistance or intolerance to prior therapy (Pfizer, 2016). Clinically recommended dose of bosutinib is 500 mg once daily under fed conditions. Bosutinib is a substrate of CYP3A4 and P-glycoprotein (P-gp) and exhibits pH-dependent aqueous solubility over the pH range of 1 to 8 with moderate in vitro passive permeability (CDER, 2012). In phase I studies, increases in bosutinib exposures, estimated as maximum plasma concentration

(C_{max}) and area under the plasma concentration-time curves (AUC), were supra-proportional at the doses of 50 to 200 mg and approximately dose-proportional at the doses of 200 to 600 mg (CDER, 2012). In contrast, bosutinib terminal half-lives were comparable between these doses (i.e., 13 to 22 hours). Since bosutinib is a substrate of P-gp, the nonlinear-to-linear pharmacokinetic profiles from lower to higher doses could be considered mainly due to a saturation of intestinal P-gp-mediated efflux on absorption, resulting in dose-dependent increases in a fraction of the dose absorbed (F_a). These findings led us to investigate the factors influencing the underlying pharmacokinetic mechanisms with PBPK models.

Bosutinib is predominantly metabolized by CYP3A4 as the primary clearance mechanism in humans with minimal urinary excretion (<2% of the administered dose) (CDER, 2012; Syed et al., 2014). For the potential DDI risk assessment as the CYP3A4 substrate, bosutinib single-dose DDI studies were conducted in healthy volunteers with coadministration of a strong CYP3A inhibitor, ketoconazole (400 mg once daily), and a strong CYP3A inducer, rifampin (600 mg once daily) (Abbas et al., 2011; Abbas et al., 2012; Abbas et al., 2015). Bosutinib exposures estimated as C_{max} and AUC increased by up to 9-fold when coadministered with ketoconazole, and decreased by ~90% when coadministered with rifampin. Accordingly, the US prescribing information advises to avoid concurrent use of bosutinib with strong or moderate CYP3A inhibitors and inducers (Pfizer, 2016). A postmarketing requirement by FDA was issued to evaluate the effect of moderate CYP3A4 inhibitors on bosutinib exposures to identify an appropriate dose when used concomitantly with moderate CYP3A inhibitors (CDER, 2012). Accordingly, based on physicochemical and pharmacokinetic parameters, we developed bosutinib PBPK models to predict clinical DDIs with less potent CYP3A inhibitors (Ono et al., 2017). The model-predicted results (2 to 4-fold) with several moderate CYP3A inhibitors were

consistent with the observed results (~2-fold) with a moderate CYP3A inhibitor, aprepitant (125 mg) (Hsyu et al., 2016). We also applied the PBPK models to predict changes in bosutinib steady-state exposures in patients with renal and hepatic impairment (Ono et al., 2017).

It has become a common practice to verify PBPK models of NMEs and refine the models, if necessary, when new datasets are available during drug development. Recently, an absolute oral bioavailability (F_{oral}) study of bosutinib was conducted in healthy subjects following an intravenous 1-hour infusion of 120 mg and an oral dose of 500 mg (Hsyu et al., 2017). However, the predicted bosutinib exposures by the previously developed PBPK model could not sufficiently match the observed results in the F_{oral} study, indicating that a further refinement of bosutinib PBPK model was required to adequately describe clinically observed results. Accordingly, we refined the previously developed bosutinib PBPK model based on the latest F_{oral} data and verified the refined PBPK model with P-glycoprotein kinetics based on the single-dose bosutinib DDI results. In addition, the verified PBPK model was applied to predict bosutinib DDIs under clinical scenarios that have not been tested. In these modeling processes, we focused on 1) understanding the contribution of intestinal P-gp -mediated efflux to bosutinib pharmacokinetics and 2) quantitatively rationalizing bosutinib DDI mechanism by ketoconazole and rifampin.

MATERIALS AND METHODS

Bosutinib Pharmacokinetic Studies in the Clinic

Detailed information about bosutinib clinical studies such as a single-dose F_{oral} study in healthy volunteers and single-dose DDI studies with ketoconazole and rifampin in healthy volunteers were previously reported (Abbas et al., 2010; Abbas et al., 2011; Abbas et al., 2012; Hsyu et al., 2017) Additional information about bosutinib pharmacokinetics is also available in the FDA website (CDER, 2012). Briefly, the F_{oral} study of bosutinib was conducted as a 2-way crossover design in healthy male subjects (n = 13 - 14) under fed conditions (Hsyu et al., 2017). A single dose of bosutinib was administered to subjects either intravenously (120 mg for 1-hour infusion) or orally (500 mg; 100 mg tablet x 5) and plasma concentrations of bosutinib were determined up to 7 days postdose. For the bosutinib DDI assessment, three single-dose studies were conducted in healthy male and female subjects with multiple-dose coadministration of ketoconazole (2 studies at bosutinib doses of 100 and 500 mg) and rifampin (1 study at bosutinib dose of 500 mg) (Abbas et al., 2011; Abbas et al., 2012; Abbas et al., 2015). In bosutinib 100-mg DDI study with ketoconazole, each subject (n = 24) received a single oral dose of 100 mg bosutinib (day 1) in a fasted state either alone (control group) or with 5-day repeated oral doses of 400 mg ketoconazole once daily on days 0 to 4 (treatment group). In bosutinib 500-mg DDI study with ketoconazole, each subject (n = 54 to 56) received a single oral dose of 500 mg bosutinib (day 1) in a fed state either alone (control group) or with 4-day repeated oral doses of 400 mg ketoconazole once daily on days 0 to 4 (treatment group). In bosutinib 500-mg DDI study with rifampin, each subject (n = 22 to 24) received a single oral dose of 500 mg bosutinib (days 1 and 14) in a fed state with 10-day repeated oral doses of 600 mg rifampin once daily

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(days 8 to 17). Plasma concentrations of bosutinib in all subjects were determined up to 72 or 96 hours postdose in these DDI studies.

Bosutinib Input Parameters in the PBPK Model

A commercially available dynamic PBPK model, Simcyp population-based simulator (version 17.1; Simcyp Ltd., Sheffield, United Kingdom), was used in the present study (Jamei et al., 2009a). First, the previously developed PBPK model was refined based on the latest F_{oral} results as mentioned before. The main differences in bosutinib input parameters between the previous and present PBPK models were hepatic microsomal intrinsic clearance (CL_{int}) and steady-state volume of distribution (V_{ss}). In addition, we utilized the advanced dissolution, absorption and metabolism (ADAM) model implemented in Simcyp to incorporate intestinal P-gp kinetic parameters into the present PBPK model. In the ADAM model, the gastrointestinal tract is divided into nine different regions, namely stomach, duodenum, jejunum I and II, ileum I, II, III and IV, and colon, as subcompartments. Physicochemical and pharmacokinetic parameters of bosutinib used for the present PBPK models are summarized in Table 1.

Input Parameters for CL

The value of CL_{int} (560 μL/min/mg protein) in the present model was back-calculated from an intravenous plasma clearance (~62 L/h) estimated in the F_{oral} study using a retrograde model implemented in Simcyp whereas that (300 μL/min/mg protein) in the previous model was estimated from a clinically observed oral clearance (~200 L/h) (Ono et al., 2017). Thus, a fraction of the dose that escapes hepatic first-pass metabolism (F_h) was estimated to be approximately ~0.5 based on the intravenous blood clearance (~0.74 L/h/kg) given that the primary clearance mechanism was CYP3A4-mediated hepatic metabolism. Since a fraction of the dose metabolized by CYP3A4 ($f_{m,CYP3A4}$) was estimated as near-unity based on the in vitro

CYP phenotyping and the human mass-balance study, the back-calculated CL_{int} values were subsequently assigned as CYP3A4-mediated metabolic CL_{int} in human liver microsomes in PBPK models. The input value of CL_{int} in human liver microsomes was scaled to CYP3A4-mediated intestinal clearance by accounting for CYP3A4 abundance in liver and intestine. Bosutinib renal clearance (CL_{renal}) was set at 1.2 L/h (~2% of systemic clearance) based the mass-balance results.

Input Parameters for V_{ss}

The V_{ss} input (28 L/kg) in the present PBPK model was a clinical estimate in the F_{oral} study whereas that in the previous model (15 L/kg) was a mean value of the predicted human V_{ss} from single-species scaling for unbound V_{ss} values ($V_{ss}/f_{u,plasma}$) from mice, rats and dogs (12 to 21 L/kg) with an exponent of unity (Ono et al., 2017). The predicted V_{ss} value by the tissue composition-based mathematical model implemented in Simcyp (as the prediction method 2) was 7.5 L/kg (Rodgers et al., 2005); therefore, K_p scalars of 2.0 and 3.7 were used to set V_{ss} inputs of 15 and 28 L/kg, respectively.

Absorption Models

A fraction of the dose absorbed (F_a) was estimated at approximately 0.7 in a single-dose human mass-balance study with [^{14}C]bosutinib at the dose of 500 mg since the recovery of bosutinib (as the parent drug) in feces was 30% of the administered dose and the fecal recovery of bosutinib was unlikely confounded by biliary excretion of the unchanged drug and/or reversible metabolites based on the metabolic profiling results in the clinical studies including mass-balance study (Abbas et al., 2010; CDER, 2012; Ono et al., 2017). Therefore, bosutinib F_a was set at 0.7 at the dose of 500 mg in the PBPK models with the first-order absorption rate constants (henceforth referred to as PBPK-F_a models). Furthermore, the ADAM model was

utilized to incorporate intestinal P-gp kinetics into bosutinib absorption (henceforth referred to as PBPK-ADAM models). It may be worth noting that P-gp kinetics can be incorporated into only the PBPK-ADAM models in Simcyp (Jamei et al., 2009b). The regional distribution of P-gp abundance along with CYP enzymes in intestine and its variability derived from meta-analysis of reported protein and mRNA values are incorporated into the population library of Simcyp (e.g., a virtual population of healthy volunteers). In order to predict bosutinib F_a, the ADAM models integrate the physicochemical and biopharmaceutical properties of bosutinib such as the pH-dependent solubility (11, 9.4, 6.1, 2.7, 0.02 and 0.053 mg/mL at pH 1, 2, 4.5, 5, 6.8 and 8, respectively) and the intestinal effective permeability (1.8×10^{-4} cm/sec) calculated from in vitro passive permeability ($\sim 7 \times 10^{-6}$ cm/sec) in low-efflux Madin-Darby canine kidney cells (Di et al., 2011; CDER, 2012). In addition, the disintegration profile was defined in the ADAM models by the 1st order kinetics with 100% maximal disintegration, a disintegration constant of 0.01 h^{-1} and a lag time of 0.25 h. Bosutinib in vitro P-gp kinetics were determined in the Caco-2 permeability study at the concentrations of 1 to 100 μM (CDER, 2012). The estimated K_m and J_{max} were 3.8 μM and 15 pmol/min, respectively, based on the kinetic model (Tachibana et al., 2010). This model assumes steady-state condition to calculate kinetic parameters; therefore, the obtained K_m value was corrected for in vitro non-specific binding (~0.1) as an extracellular nonspecific binding, resulting in the unbound K_m of 0.38 μM as an input parameter. These calculations were performed with GraphPad Prism 6 (GraphPad Software Inc., San Diego, CA). In the PBPK-ADAM models, an in vitro-to-in vivo scaling factor (SF) for P-gp J_{max} was optimized to adequately recover the observed results whereas K_m was fixed assuming that was intrinsic.

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PBPK Simulation by Simcyp

To understand bosutinib pharmacokinetics, our modeling and simulation approaches are practically categorized into three main tiers: 1) model refinement based on the latest F_{oral} data, 2) model verification based on the single-dose bosutinib DDI results, and 3) model application to predict bosutinib DDIs under possible scenarios that have not been tested clinically. In these processes, sensitivity analysis and optimization (SAO) for the model input parameters such as $f_{u,gut}$ and SFs for P-gp J_{max} were performed as the model refinement and verification. Outlines of the PBPK modeling and simulation are summarized in Table 2 along with key parameters explored.

Simulation Outlines

Simulation of all clinical trials in Simcyp was performed with a virtual population of healthy volunteers in 6 trials of 6 subjects (total 36 subjects), each aged 20 to 50 years with a female/male ratio of 0.5, whose CYP3A4 degradation rate constant (k_{deg}) was 0.019 h^{-1} in liver and 0.030 h^{-1} in intestine. The output sampling interval in Simcyp simulation tool box was set to 0.2 hours in all simulations. In the PBPK-ADAM models, the gastric emptying time in virtual populations was modified from the default values of 0.4 hour in fasted and 1 hour in fed to the maximal values of 2 and 4 hours, respectively, to sufficiently adapt clinically observed t_{max} (4 to 6 hours) (CDER, 2012). It has been reported that the mean gastric emptying time was 15.3 ± 4.7 h (4.3 to 20 h) in healthy subjects (n = 19) following the standard high fat meal recommended by the FDA guidance (Koziolek et al., 2015). In the F_{oral} and DDI studies, the study outlines of all simulation were based on the clinical study designs described above. For model application, a single oral dose of bosutinib 500 mg was administered to a virtual population of healthy volunteers on day 5 with and without 16-day repeated oral administration

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of dual CYP3A/P-glycoprotein inhibitors, itraconazole (200 mg once daily) and verapamil (80 mg three times a day).

DDI Prediction on P-gp

Regarding DDIs on intestinal P-gp-mediated efflux, ketoconazole was assumed to inhibit P-gp-mediated efflux in a competitive manner. The reported ketoconazole in vitro IC_{50} values against P-gp varied ~200-fold (a median of 2.0 μM with a range of 0.24 to 49 μM using various substrates) or ~11-fold (a median of 1.5 μM with a range of 0.42 to 4.6 μM using digoxin as a substrate) in the Metabolism and Transport Drug Interaction Database (DIDB) (School of Pharmacy, University of Washington, Seattle, WA). Therefore, the SAO for ketoconazole K_i were performed with PBPK-ADAM models to adequately recover clinical DDI results. Rifampin was assumed to increase intestinal P-gp abundances in PBPK-ADAM models since it was reported that multiple-dose administration of rifampin increased the intestinal P-gp abundances by 3.5-fold similar to intestinal CYP3A4 by 4.5-fold (Greiner et al., 1999). Another report also indicated that multiple-dose administration of rifampin increased intestinal P-gp expression (mRNA) and abundance (protein) by 3- and 8-fold, respectively (Giessmann et al., 2004). However, there is no function in Simcyp for precipitant drug-mediated increases in P-gp abundances. Therefore, SFs for P-gp J_{max} were assumed to increase with increasing intestinal P-gp abundances by rifampin-mediated induction. This also assumed that increases in intestinal P-gp abundances were proportional to increases in P-gp mediated efflux activities. Accordingly, the SAO for P-gp J_{max} SFs (for rifampin-mediated P-gp induction) were performed to adequately recover clinical results.

For bosutinib DDI predictions, the vendor-verified compound files in Simcyp library were used, i.e., ketoconazole (sim-ketoconazole 400 mg QD), rifampin (sv-rifampicin-md),

itraconazole (sv-itraconazole_fed capsule), itraconazole metabolite (sv-OH-itraconazole), verapamil (sv-verapamil) and verapamil metabolite (sv-norverapamil). The input parameters on CYP3A4-mediated DDIs in these compound files were as follows: ketoconazole competitive K_i = 0.015 μM ($f_{u,mic}$ = 0.97); rifampin induction E_{max} = 16, EC_{50} = 0.32 μM and competitive K_i = 15 μM ($f_{u,mic}$ = 1), itraconazole competitive K_i = 0.0013 μM ($f_{u,mic}$ = 1); itraconazole metabolite competitive K_i = 0.0023 μM ($f_{u,mic}$ = 1); verapamil mechanism-based inhibition K_I = 2.21 μM ($f_{u,mic}$ = 1) and k_{inact} = 2 h^{-1}); verapamil metabolite mechanism-based inhibition K_I = 10.3 μM ($f_{u,mic}$ = 1) and k_{inact} = 18 h^{-1}). The input parameters of intestinal P-kinetics were as follows: verapamil K_m = 0.734 μM ($f_{u,mic}$ = 1), J_{max} = 2.814 pmol/min and K_i = 0.16 μM ($f_{u,mic}$ = 1); verapamil metabolite K_i = 0.04 μM ($f_{u,mic}$ = 1). For itraconazole-mediated P-gp inhibition, no P-gp inhibition parameters were incorporated into the default compound file; therefore, based on the reported values (a median of 1.7 μM with a range of 0.45 to 6.7 μM using digoxin as a substrate) in the DIDB, the lower end of itraconazole P-gp K_i value was used as the input parameter (i.e., 0.5 μM). P-gp K_i value of verapamil (0.16 μM) in the default compound file was also near the lower end of reported values (a median 4.9 μM with a range of 0.06 to 57 μM using digoxin as a substrate with an exception of 224 μM) in the DIDB.

Data Analysis

Pharmacokinetic parameters such as C_{max}, time to reach C_{max} (t_{max}) and AUC from time zero to infinity and the ratios of C_{max} ($C_{max}R$) and AUC ($AUCR$) in treatment groups relative to control groups were obtained from Simcyp outputs. To evaluate predictive model performance, the ratios of predicted-to-observed pharmacokinetic parameters (P/O) were calculated according to the following equation:

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$$P/O = \frac{Predicted\ Parameters}{Observed\ Parameters}$$

To assess the predictive model performance, the P/O ratios within ±50% of the observed results (i.e., 0.67 to 1.5) were provisionally considered to be acceptable as the predefined criteria (Guest et al., 2011; Sager et al., 2015).

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RESULTS

Bosutinib PBPK Models without P-gp Kinetics

Model Refinement on Intravenous Pharmacokinetics

Based on the intravenous plasma concentration-time profiles in healthy subjects at the dose of 120 mg, we compared the predictive performance of the PBPK models between 2 different parameter sets, i.e., CL_{int} of 300 μL/min/mg protein and V_{ss} of 15 L/kg used in the previous model and CL_{int} of 560 μL/min/mg protein and V_{ss} of 28 L/kg obtained from the F_{oral} study. The intravenous plasma concentration-time profiles were over-predicted by the PBPK model with the parameters used in the previous model (Figure 1A), resulting in that C_{max} and AUC values were higher than the observed values with the P/O ratios of 1.9 and 1.5, respectively (Table 3). In contrast, the predicted P/O ratio for AUC was within 10% of the observed values in the PBPK model with the parameters from the F_{oral} study whereas that for C_{max} (~60%) was close to the acceptable range (≤±50%) (Table 3 and Figure 1B). Accordingly, the PBPK model was refined with the CL_{int} of 560 μL/min/mg protein and the V_{ss} of 28 L/kg obtained from the F_{oral} study.

Model Refinement on Oral Pharmacokinetics

The oral plasma concentration-time profiles of bosutinib in healthy subjects at the dose of 500 mg were predicted by the refined PBPK-F_a model with fixed F_a of 0.7 and $f_{u,gut}$ of 1 used in the previous PBPK model. The PBPK-F_a model considerably under-predicted the plasma concentration-time profiles (Figure 1C), resulting in that the predicted C_{max} and AUC values were ≥2-fold lower than the observed values (Table 3). Since bosutinib F_a was estimated at 0.7 in the mass-balance study, the SAO for $f_{u,gut}$ ranging from 0.01 to 1 was performed to investigate the effect of $f_{u,gut}$ on overall outcomes, particularly, a fraction of the dose that escapes intestinal

first-pass metabolism (F_g). Results showed that the plasma concentration-time profiles were reasonably predicted by the PBPK-F_a models with $f_{u,gut}$ of 0.01 to 0.1 (≤20% difference in AUC). Accordingly, $f_{u,gut}$ was assumed to be comparable to $f_{u,plasma}$ of 0.063 (Table 3 and Figure 1D). The difference between $f_{u,gut}$ of 0.063 and 1 resulted in an approximately 2-fold difference in the predicted F_g (median) of 0.93 and 0.45, respectively.

Model Verification on DDI Outcomes

Bosutinib single-dose DDIs with ketoconazole and rifampin were predicted by the refined PBPK-F_a models with $f_{u,gut}$ of 0.063. The F_a value was set at 0.7 at bosutinib dose of 500 mg whereas that was calculated at 0.3 at the doses of 100 mg based on the comparison of AUC estimates between 100 and 500 mg. The predicted C_{max} and AUC were within ±35% of the observed results in control groups (bosutinib alone) from these 3 DDI studies (Table 4, Table 5 and Table 6). In contrast, at bosutinib 100 mg with ketoconazole, the PBPK-F_a model considerably under-predicted the plasma concentrations of bosutinib in treatment group (bosutinib with ketoconazole) with the P/O ratios of 0.5 for both C_{max} and AUC (Table 4). The P/O ratios for C_{max}R and AUCR were approximately 0.6. Thus, the PBPK-F_a model significantly under-predicted the effect of ketoconazole on bosutinib exposures at the dose of 100 mg (Figure 2A). At bosutinib 500 mg with ketoconazole, the PBPK-F_a model tended to under-predicted the plasma concentrations of bosutinib in treatment group with the P/O ratios of 0.70 and 0.88 for C_{max} and AUC, respectively (Table 5 and Figure 2B). The P/O ratios of C_{max}R and AUCR were 0.97 and 0.66, respectively. Thus, the under-prediction was more pronounced at the dose of 100 mg than 500 mg in the DDI studies with ketoconazole. At bosutinib 500 mg with rifampin, the PBPK-F_a model significantly over-predicted the plasma concentrations of bosutinib with rifampin, resulting in the P/O ratios of 1.6 for C_{max} and 2.4 for AUC (Table 6 and

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Figure 2C). The predicted $C_{max}R$ and AUCR were approximately 2-fold higher than the observed ratios, i.e., the P/O ratios of 1.9 for both $C_{max}R$ and AUCR.

In these DDI predictions, the predicted F_h values in control groups were approximately 0.6 (median), which increased to near-unity (~1.0) by ketoconazole-mediated CYP3A4 inhibition and decreased to ~0.1 by rifampin-mediated CYP3A4 induction. Thus, the modeling results suggested that hepatic metabolism of bosutinib was near-completely inhibited by ketoconazole or induced by rifampin. The predicted F_g values (~0.9) in control groups also increased to near-unity (~1.0) by ketoconazole, suggesting that the contribution of intestinal metabolism to systemic DDIs was minimal. The predicted F_g values in the DDI study with rifampin decreased to ~0.6, which appeared to be constrained by the faster CYP3A4 degradation rates in intestine (0.03 h^{-1}) than liver (0.0193 h^{-1}) and the small $f_{u,gut}$ (0.063) optimized by the aforementioned SAO. Overall, the modeling results suggested that the predicted changes in bosutinib F_h and F_g by ketoconazole and rifampin could not sufficiently recover the observed DDI results. Therefore, these precipitant drugs could likely impact the extent of bosutinib absorption (F_a) through P-gp-mediated efflux in intestine.

Bosutinib PBPK Models with P-gp Kinetics

Model Refinement on Oral Pharmacokinetics

To incorporate bosutinib P-gp kinetics into the intestinal absorption process, the PBPK-ADAM models were utilized with the refined bosutinib parameters. For bosutinib P-gp kinetic parameters, in vitro K_m (0.38 μM) and J_{max} (15 pmol/min) determined in Caco-2 cells were initially incorporated into the PBPK-ADAM models. Clinical studies used for the model refinement were an oral group of bosutinib F_{oral} study at the dose of 500 mg and control groups of DDI studies with ketoconazole (100 and bosutinib 500 mg) and rifampin (bosutinib 500 mg).

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The model-predicted plasma concentration-time profiles of bosutinib in control group at the dose of 100 mg were considerably over-predicted by the PBPK-ADAM model with P-gp inputs, resulting in the P/O ratios of 4.5 and 2.0 for C_{max} and AUC, respectively (Table 4 and Supplemental Figure S1A). The predicted F_a was approximately 0.7 (median). When compared to the predicted results by the PBPK-ADAM model without P-gp kinetics, the over-prediction was slightly improved from the P/O ratios of 5.4 and 2.2 for C_{max} and AUC, respectively (Table 4). Following the SAO for J_{max} SFs in PBPK-ADAM models, the predicted C_{max} and AUC with a SF of 25 were within ±20% of the observed results (Table 4 and Supplemental Figure S2A). The predicted F_a was ~0.3, which was ~3-fold lower than that (~0.7) by the PBPK-ADAM model with the SF of unity.

At bosutinib dose of 500 mg, the PBPK-ADAM model with J_{max} SF of unity sufficiently predicted the plasma concentration-time profiles of bosutinib in an oral group of bosutinib F_{oral} study and control groups of bosutinib 500 mg DDI studies (Supplemental Figure S1). The P/O ratios were 1.1 to 1.2 for C_{max} and 1.0 to 1.4 for AUC (Table 3, Table 5 and Table 6). The model performance was slightly improved from the PBPK-ADAM model without P-gp parameters (P/O ratios of 1.3 to 1.4 for C_{max} and 1.1 to 1.6 for AUC). Thus, the effects of P-intestinal gp-mediated efflux on bosutinib exposures could be minimal at clinically recommended dose of 500 mg. The SAO for J_{max} suggested that the predictive model performance could be improved further to the P/O ratios of 0.95 to 1.0 for C_{max} and 0.94 to 1.2 for AUC when J_{max} SF was set at 2 in the F_{oral} study and at 4 in the DDI studies (Table 3, Table 5, Table 6 and Supplemental Figure S2). Subsequently, J_{max} SF of 4 was used for the following model verification based on DDI results. The difference in bosutinib exposures at the dose of 500 mg among these studies could be considered to be within the variability between clinical studies including inter-

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individual variability. The predicted F_a in these studies was 0.5 to 0.7, which was comparable to the estimated F_a of 0.7 in the mass-balance study.

Model Verification on DDI Outcomes

The DDI prediction of bosutinib with ketoconazole was performed by the PBPK-ADAM models with the optimized J_{max} SFs at the doses of 100 and 500 mg. As suggested by the PBPK-F_a modeling results, the effect of ketoconazole on bosutinib exposures was under-predicted by the PBPK-ADAM model without ketoconazole-mediated P-gp inhibition (Figure 3A and 3C), particularly at the dose of 100 mg. The P/O ratios for C_{max}R and AUCR were 0.47 and 0.66, respectively, at 100 mg (Table 4), whereas those were 0.72 and 0.60, respectively, at 500 mg (Table 5). The predicted F_h and F_g in control groups increased to near-unity in treatment groups with ketoconazole. Thus, the modeling results suggested that the hepatic and intestinal metabolism of bosutinib was near-completely inhibited by ketoconazole; yet the PBPK-ADAM models under-predicted the effect of ketoconazole on bosutinib exposures. Accordingly, ketoconazole K_i for P-gp was incorporated into the PBPK-ADAM models to account for ketoconazole-mediated P-gp inhibition. Following the SAO for ketoconazole K_i, the predicted C_{max} and AUC by PBPK-ADAM models with K_i values of 0.1 to 0.3 μM were in the acceptable range (≤±50%) of the observed results in treatment group at bosutinib dose of 100 mg. When K_i was set at 0.2 μM, the predicted C_{max} and AUC were within ±25% of the observed results, resulting in the P/O ratios for C_{max}R and AUCR of 1.0 and 1.3, respectively (Table 4 and Figure 3B). Bosutinib F_a (median) was predicted to increase from 0.2 to 0.4. Assuming the general hypothesis that K_i was intrinsic, the effect of ketoconazole on bosutinib oral exposures at the dose of 500 mg was predicted by the PBPK-ADAM models with ketoconazole K_i of 0.2 μM. The predicted C_{max} and AUC were within ≤±20% of the observed values, resulting in the P/O

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ratios for $C_{max}R$ and AUCR of 0.87 and 0.70, respectively (Table 5 and Figure 3D). Compared to the predicted results without ketoconazole K_i, the DDI prediction was slightly improved from the P/O ratios for $C_{max}R$ of 0.72 and AUCR of 0.60. Bosutinib F_a (median) was predicted to increase from 0.5 to 0.7 at 500 mg by ketoconazole-mediated P-gp inhibition.

The DDI prediction of bosutinib with rifampin was also performed by the PBPK-ADAM model with the optimized intestinal P-gp J_{max} SF. The PBPK-ADAM model considerably under-predicted the effect of rifampin on bosutinib exposures even though the predicted F_h and F_g markedly decreased to 0.11 and 0.64, respectively (Figure 4A). The P/O ratios for C_{max} and AUC were 2.6 to 3.0, resulting in the P/O ratios of 2.5 to 2.8 for $C_{max}R$ and AUCR (Table 6). The increases in intestinal P-gp abundances were therefore incorporated into PBPK-ADAM models to account for rifampin-mediated P-gp induction as was suggested by the PBPK-F_a modeling results. Following the SAO for J_{max} SFs as rifampin-mediated fold-increases in P-gp abundances, the predicted C_{max} and AUC were in the acceptable range ($\leq\pm$50%) of the observed results in treatment group when J_{max} SFs were set at 32 to 48 corresponding to P-gp induction of 8 to 12-fold on top of the SF of 4 used for control group (Supplemental Table S1). Assuming the fold-increase in P-gp abundance of 10, the C_{max} and AUC with were within ±30% of the observed values, resulting in the P/O ratios for $C_{max}R$ and AUCR were 0.86 and 0.95, respectively (Figure 4B and Table 6). Bosutinib F_a was predicted to decrease from 0.6 to 0.2 by rifampin-mediated P-gp induction.

Model Application to DDI Prediction

Bosutinib DDIs with dual CYP3A/P-gp inhibitors, itraconazole and verapamil, were predicted by the PBPK-ADAM models. In these DDI predictions, a single oral dose of bosutinib 500 mg was administered to a virtual population of healthy volunteers on day 5 with and without

16-day repeated oral administration of either itraconazole (200 mg one daily) or verapamil (80 mg three times a day). The predicted $C_{max}R$ and AUCR were 2.0 and 8.5, respectively, with itraconazole and 2.0 and 5.1, respectively, with verapamil (Table 7). Compared to the DDI prediction without P-gp inhibition (i.e., only CYP3A inhibition), the differences in $C_{max}R$ and AUCR were negligible to minimal. These results together with the DDI prediction with ketoconazole suggested minimal impacts of P-gp-mediated efflux on bosutinib DDIs with P-gp inhibitors at clinically recommended dose of bosutinib 500 mg.

DISCUSSION

Utilizing PBPK modeling for understanding pharmacokinetic mechanism of NMEs becomes common practices in drug development as well as regulatory decision-making (Rowland et al., 2011; Huang and Rowland, 2012; Prueksaritanont et al., 2013; Wagner et al., 2015; Wagner et al., 2016; Shebley et al., 2018). Modeling approaches typically consist of three main tiers, model development, verification and application. Subsequently, it is critical to continuously verify and refine PBPK models, if necessary, based on latest available data. Accordingly, we have refined the previously developed bosutinib PBPK model with the latest F_{oral} results. Apparently, the present PBPK model could rationalize the underlying DDI mechanisms with ketoconazole and rifampin through not only CYP3A4 but also P-gp. However, the present study undoubtedly highlighted the challenges of PBPK modeling for P-gp substrate drugs. Some potential issues raised in the present study therefore remain and warrant further discussion.

One of the most important pharmacokinetic parameters for oral drugs is F_{oral} ($F_a \times F_g \times F_h$). Bosutinib F_{oral} and F_h were estimated at ~0.3 and ~0.5, respectively, in the F_{oral} study and F_a was estimated at ~0.7 in the mass-balance study at the dose of 500 mg. Subsequently, the calculated F_g was ~0.9 from F_{oral} (~0.3), F_a (~0.7) and F_h (~0.5). Thus, the F_{oral} result was valuable to verify the PBPK models. However, the refined PBPK-F_a models under-predicted the effects of ketoconazole and rifampin on bosutinib exposures. In both cases, the observed DDI results could not be recovered sufficiently by the model-predicted changes in bosutinib F_g and F_h as mentioned before, suggesting that bosutinib F_a could possibly be altered by these precipitant drugs through P-gp mediated efflux. Consistently, the increases in bosutinib exposures estimated as C_{max} and AUC were supra-proportional at the lower doses of 50 to

200 mg and approximately dose-proportional at the higher doses of 200 to 600 mg (CDER, 2012). In contrast, the observed terminal half-lives (13 to 22 hour) were comparable across the doses, suggesting the linear elimination (e.g., hepatic clearance) across the doses tested. This finding appeared to be consistent with in vitro metabolism data showing much higher K_m estimates of two major metabolites (8 to 23 μM for oxydechlorinated and N-desmethyl bosutinib) than the observed unbound steady-state C_{max} of ~0.03 μM (CDER, 2012). Thus, bosutinib nonlinear pharmacokinetics could likely result from the dose-dependent increases in F_a due largely to a saturation of intestinal P-gp efflux.

Two of the most important factors governing F_a are solubility and permeability including active transports. It has been reported that it would be challenging to accurately predict F_a of many drugs, particularly, basic compounds with low solubility (Zhang et al., 2014; Li et al., 2017; Lin and Wong, 2017). Bosutinib exhibits pH-dependent aqueous solubility with the solubility decreasing from 21 to 0.038 mM over the pH range of 1 to 6.8. In contrast, bosutinib intestinal concentrations calculated by dose amounts divided by 250 mL were 0.38 to 3.8 mM at the doses of 50 to 500 mg. Therefore, bosutinib F_a could be limited by the solubility whereas its distinctive pH-dependent solubility could potentially increase in gastrointestinal solubility in vivo. Bosutinib exhibited positive food effects (high fat meal) on oral exposures (~1.6-fold) in healthy volunteers (n = 23 - 24) at the dose of 400 mg (CDER, 2012). The observed positive food effects were sufficiently predicted by the present PBPK-ADAM model showing ~1.5-fold higher exposures in a fed state (high fat meal) than a fasted state (Supplemental Table S2). Thus, the modeling results suggested that the PBPK-ADAM model could adequately predict the solubility-limited absorption. Bosutinib in vitro passive permeability was moderate (~7×10^{-6} cm/sec) which was on the borderline of the proposed cut-off (5×10^{-6} cm/sec) on F_a in

the extended clearance classification system (Di et al., 2011; Varma et al., 2012). The relative P-gp distribution increases from proximal to distal small intestine while the expression levels appear slightly higher in jejunum than ileum (Fricker et al., 1996; Mouly and Paine, 2003; Englund et al., 2006; Harwood et al., 2015). Therefore, highly soluble and permeable drugs, those are even P-gp substrates, can be absorbed rapidly and extensively in duodenum and proximal jejunum. In contrast, P-gp-mediated efflux frequently reduces absorption rate and extent of P-gp substrates having low-to-moderate solubility or permeability. Consistently, bosutinib absorption was relatively slow with the observed median t_{max} of 4 to 6 hours, and its F_a was incomplete (≤0.7) at the doses tested. Unbound intracellular enterocyte concentrations calculated by k_a (0.61 h^{-1}), F_a (1), $f_{u,gut}$ (0.063) and enterocyte blood flow (18 L/h) were 0.2 to 2.0 μM at the doses of 50 to 500 mg (Rostami-Hodjegan and Tucker, 2004). When the predicted F_a values (0.3 to 0.7) were used, the calculated unbound intracellular enterocyte concentrations were 0.06 to 1.4 μM. In the ADAM model, bosutinib enterocyte concentrations were predicted in each region (subcompartment) of GI tract as a function of time. The predicted maximal concentrations in the subcompartments were 0.1 to 0.7 μM and 0.8 to 2.4 μM at the doses of 100 and 500 mg, respectively. Thus, the predicted enterocyte concentrations were in a comparable range of the in vitro K_m (0.38 μM). Collectively, these findings suggested a potential interplay between the pH-dependent solubility, moderate permeability and P-gp-mediated efflux on bosutinib absorption, as was also suggested for other P-gp substrates (Burton et al., 2002; Jamei et al., 2009b; Sjogren et al., 2013).

SAO are powerful tools to assess effects of uncertainty around input parameters on overall outputs, often leading to model improvement with further providing mechanistic insights (Zhao et al., 2012; Shardlow et al., 2013; Shepard et al., 2015). Accordingly, the SAO for bosutinib P-

gp kinetic parameters were performed in the present study. First, bosutinib K_m was fixed assuming that was intrinsic. Consequently, J_{max} SFs were optimized to adequately recover the observed results. The SAO revealed the dose-dependent decrease in SFs at the doses of 100 to 500 mg to recover the observed results, suggesting the dose-dependent decrease in P-gp-mediated CL_{int} in intestine. The general hypothesis is that J_{max} is consistent across doses. Thus, the difference in J_{max} SFs between the doses suggested that J_{max} SFs could be optimized as *apparent* J_{max} instead of *true* J_{max}. One of the potential reasons could be that PBPK models might not adequately capture the interplay between P-gp-mediated efflux, permeability and pH-dependent solubility in each region of GI tract, e.g., the regional differences in P-gp meditated CL_{int}. Further model refinement may be required to recover nonlinear pharmacokinetics of bosutinib across doses, suggesting the present PBPK models could be considered to be "fit-for-purpose" models.

Ketoconazole is generally assumed to be only an inhibitor of CYP3A in clinical DDI studies although ketoconazole is known to inhibit P-gp (Rautio et al., 2006; Vermeer et al., 2016). The reason behind it could likely be due to minimal P-gp effects on the absorption of many dual CYP3A and P-gp substrates because of the saturation of P-gp-mediated efflux at clinical doses. Clinically observed bosutinib $C_{max}R$ and AUCR by ketoconazole was more pronounced at the dose of 100 mg than 500 mg, which appeared to be consistent with the dose-dependent saturation of P-gp-mediated efflux (Table 4 and Table 5). To adequately recover the observed DDIs, the SAO indicated ketoconazole in vivo K_i of 0.1 to 0.3 μM, which were in line with the lower end of the reported in vitro IC_{50} of ~0.2 μM in the DIDB. The K_i values for itraconazole and verapamil used in the PBPK models were also near the lower end of reported values as noted

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above. Overall, the PBPK modeling results suggested minimal impacts of P-gp-mediated efflux on bosutinib DDIs with P-gp inhibitors at clinically recommended dose of 500 mg.

Rifampin is well-known to be a modulator for not only CYP enzymes but also transporter proteins including P-gp (Haslam et al., 2008; Williamson et al., 2013; Wagner et al., 2016). Following multiple-dose coadministration of rifampin, oral exposures of a P-gp probe substrate, digoxin, decreased by ~2-fold whereas intravenous exposures were not significantly altered (Novi et al., 1980; Gault et al., 1984; Greiner et al., 1999). Thus, the decrease in oral exposure of digoxin was likely due to rifampin-mediated P-gp induction in intestine, resulting in the decrease in digoxin F_a. Since bosutinib was a dual-substrate of CYP3A4 and P-gp, the decrease in bosutinib exposures by rifampin could possibly be caused by complex DDI mechanisms through not only CYP3A4 but also P-gp. Consistently, the effect of rifampin on bosutinib exposures was considerably under-predicted by the PBPK models when accounting for only rifampin-mediated CYP3A4 induction. The subsequent SAO suggested that the observed DDI results could adequately be recovered by 8 to 12-fold increases in intestinal P-gp abundances by rifampin (Table 6). The predicted fold-increases in intestinal P-gp abundances were comparable to the reported results (Giessmann et al., 2004). The predicted increases in P-gp abundances were also comparable to those in CYP3A4 induction (Almond et al., 2016), which appeared to be consistent with the literature reporting the similar increases in rifampin-mediated CYP3A4 and P-gp expression levels (Greiner et al., 1999). For DDI prediction with CYP3A inducers, efavirenz is frequently being used for PBPK modeling as a moderate inducer (Ke et al., 2016; Wagner et al., 2016). However, it has been reported that efavirenz did not induce intestinal P-gp in the clinic (Mouly et al., 2002; Oswald et al., 2012). Provisionally, the results of bosutinib DDI prediction with rifampin using the different fold-increases in intestinal P-gp abundance (i.e.,

1 to 16-fold) could possibly be used as indexes of DDI prediction with CYP3A/P-gp dual inducers (Supplemental Table S1). With increasing P-gp abundances by 16-fold, bosutinib $C_{max}R$ and AUCR decreased from 0.34 to 0.09 and 0.21 to 0.06, respectively, suggesting some degree of impacts of intestinal P-gp induction on bosutinib exposures at clinically recommended dose of 500 mg.

In summary, the present study demonstrated that bosutinib PBPK models were reasonably refined and verified based on the currently available data. The results suggested that P-gp-mediated intestinal efflux could play a substantial role on bosutinib DDIs with ketoconazole and rifampin. Overall, it would be critical to incorporate P-gp kinetics in the PBPK models to understand the underlying DDI mechanisms for P-gp substrates such as bosutinib, particularly when clinical data exhibited nonlinear pharmacokinetics that could be due to a saturation of intestinal P-gp-mediated efflux.

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Authorship Contribution

Participated in research design: Yamazaki

Conduct experiments: na

Performed data analysis: Costales, Kimoto and Yamazaki

Wrote or contribute to the writing of the manuscript: Costales, Kimoto, Loi, Varma and Yamazaki

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Footnotes

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Legends for Figures

Figure 1. Clinically observed and PBPK model-predicted plasma concentrations of bosutinib in healthy subjects after a single intravenous and oral administration. Bosutinib was administered intravenously (120 mg for 1-h infusion) and orally (500 mg) to healthy subjects in a single-dose crossover study. Bosutinib plasma concentrations were predicted in a virtual population of healthy subjects with PBPK-F_a models. Input parameters of bosutinib PBPK models were CL_{int} of 300 μL/min/mg protein and V_{ss} of 15 L/kg (A) or CL_{int} of 560 μL/min/mg protein and V_{ss} = 28 L/kg (B, C & D) with $f_{u,gut}$ of 1 (C) or 0.063 (D). The observed and predicted plasma concentrations are expressed as mean ± SD (○) and mean (—) with 5th and 95th percentiles (---), respectively.

Figure 2. Clinically observed and PBPK model-predicted plasma concentrations of bosutinib in healthy subjects after a single oral administration of bosutinib with and without coadministration of ketoconazole and rifampin. A single oral dose of bosutinib was administered to healthy subjects at the dose of 100 mg (A) or 500 mg (B & C) with and without repeated coadministration of ketoconazole 400 mg once daily (A & B) or rifampin 600 mg once daily (C). Bosutinib plasma concentrations were predicted in a virtual population of healthy subjects with PBPK-F_a models. The observed and predicted plasma concentrations are expressed as mean ± SD (○) and mean (—) with 5th and 95th percentiles (---), respectively.

Figure 3. **Clinically observed and PBPK model-predicted plasma concentrations of bosutinib in healthy subjects after a single oral administration of bosutinib with and without coadministration of ketoconazole.** A single oral dose of bosutinib was administered to healthy subjects at the dose of 100 mg (A & B) or 500 mg (C & D) with and without repeated

coadministration of 400 mg ketoconazole once daily. Bosutinib plasma concentrations were predicted in a virtual population of healthy subjects with PBPK-ADAM models. Input parameters of P-gp kinetic parameters in PBPK models were bosutinib K_m of 0.38 μM and J_{max} of 15 pmol/min with J_{max} SFs of 25 (A & B) or 4 (C & D) without ketoconazole K_i (A & C) or with ketoconazole K_i of 0.2 μM (B & D). The observed and predicted plasma concentrations are expressed as mean ± SD (○) and mean (—) with 5^{th} and 95^{th} percentiles (---), respectively.

Figure 4. **Clinically observed and PBPK model-predicted plasma concentrations of bosutinib in healthy subjects after a single oral administration of bosutinib with and without coadministration of rifampin.** A single oral dose of bosutinib was administered to healthy subjects at the dose of 500 mg with and without repeated coadministration of 600 mg rifampin once daily. Bosutinib plasma concentrations were predicted in a virtual population of healthy subjects with PBPK-ADAM models. Input parameters of P-gp kinetic parameters in bosutinib PBPK models were K_m of 0.38 μM and J_{max} of 15 pmol/min with J_{max} SFs of 4 (A) or 40 (B). The observed and predicted plasma concentrations are expressed as mean ± SD (○) and mean (—) with 5^{th} and 95^{th} percentiles (---), respectively.

Table 1

Physicochemical and pharmacokinetic parameters of bosutinib used for PBPK model-simulation

Parameter (units)	Value	Source
Molecular weight	530	Calculated
LogP	3.1	Measured
pK_a (monobase)	7.9	Measured
$f_{u,plasma}$	0.063	Measured in vitro
B/P	1.2	Measured in vitro
F_a [a]	0.3 – 0.7	Mass-balance study results
k_a (h^{-1}) [a]	0.13	Clinical study results
Lag time (h) [a]	1	Clinical study results
Solubility (mg/mL)	0.02 – 11 (at pH 1 – 8)	Measured in vitro
$P_{eff,man}$ (10^{-4} cm/sec)	1.8	Calculated from physiochemical property
Q_{gut} (L/h) [a]	8.7	Calculated by Simcyp
$f_{u,gut}$	0.063	Predicted by sensitivity analysis
V_{ss} (L/kg)	28	Clinical study results
K_p scalar	3.7	Adjusted to predict the observed V_{ss} value
$CL_{int,CYP3A4}$ (μL/min/mg protein) [b]	560	Back-calculated in Simcyp
CL_{renal} (L/h)	1.2	Clinical study results
P-gp K_m (μM) [c]	0.38	Measured in Caco-2 cells
P-gp J_{max} (pmol/min) [c]	15	Measured in Caco-2 cells
P-gp J_{max} SF [c]	1 – 25	Optimized to recover the observed results

[a] Input parameters used for PBPK-F_a models. [b] Huma liver microsomal CL_{int} back-calculated from the clinically observed clearance (~62 L/h) using a retrograde model implemented in Simcyp. [c] Intestinal P-pg parameters used for PBPK-ADAM models.

Table 2

Outlines of bosutinib PBPK modeling for model refinement, verification and application

PBPK model [a]	Approach	Bosutinib dose (mg)	Precipitant drug	Clinical studies used	Key parameters explored
IV	Refinement	120 [b]	–	F_{oral}	CL_{int} & V_{ss}
F_a	Refinement	500	–	F_{oral}	$f_{u,gut}$
	Verification	100 & 500	Ketoconazole	DDI	C_{max}R & AUCR
		500	Rifampin	DDI	C_{max}R & AUCR
ADAM	Refinement	500	–	F_{oral}	P-gp kinetics (J_{max} SF) [c]
		100 & 500	–	DDI [d]	P-gp kinetics (J_{max} SF) [c]
	Verification	100 & 500	Ketoconazole	DDI	P-gp inhibition (K_i) [e]
		500	Rifampin	DDI	P-gp induction (abundance) [f]
	Application	500	Itraconazole	–	DDI prediction
		500	Verapamil	–	DDI prediction

[a] PBPK model without absorption model (PBPK-IV), PBPK model with the 1st order absorption model (PBPK-F_a) and PBPK model with the ADAM model using P-gp kinetic parameters (PBPK-ADAM). [b] Single intravenous 1-h infusion; [c] in vitro-to-in vivo scaling factors for intestinal P-gp J_{max}. [d] Control groups (bosutinib alone) of the DDI study with ketoconazole (100 and 500 mg) and rifampin (500 mg). [e] Ketoconazole K_i on intestinal P-gp. [f] Rifampin-mediated increases in intestinal P-gp abundance. –, not applicable.

Table 3

Clinically observed and PBPK model-predicted pharmacokinetic parameters of bosutinib in humans following a single intravenous and oral administration of bosutinib

Dose *mg*	PBPK model [a]	J_{max} [b] SF	Analysis [c]	C_{max} *ng/mL*	t_{max} *h*	AUC *ng·h/ml*
120	–	–	Obs	347 (28)	1 (1 – 1)	1920 (26)
	IV-1	–	Pred	662 (14)	1 (1 – 1)	2934 (35)
			P/O	1.91	–	1.53
	IV-2	–	Pred	563 (13)	1 (1 – 1)	2128 (28)
			P/O	1.62	-	1.11
500	–	–	Obs	109 (43)	6 (2 – 8)	2736 (44)
	F_a-1	–	Pred	36 (55)	4 (3 – 7)	1012 (77)
			P/O	0.33	–	0.37
	F_a-2	–	Pred	83 (47)	4 (3 – 7)	2374 (46)
			P/O	0.76	–	0.87
	ADAM	–	Pred	138 (48)	5 (2 – 11)	3038 (64)
			P/O	1.27	–	1.11
		1	Pred	123 (49)	5 (2 – 15)	2770 (66)
			P/O	1.12	–	1.01
		2	Pred	112 (51)	5 (2 – 17)	2560 (68)
			P/O	1.03	–	0.94
		4	Pred	96 (53)	5 (2 – 20)	2235 (71)
			P/O	0.88	–	0.82

Data are expressed as geometric mean with percent coefficient of variation (CV%) in parentheses (n = 13-14 for the observed; n = 6 per group × 6 groups for the predicted) except for median t_{max} with minimal to maximal values.

[a] CL_{int} = 300 μL/min/mg protein & V_{ss} = 15 L/kg (IV-1) or CL_{int} = 560 μL/min/mg protein & V_{ss} = 28 L/kg (IV-2, F_a-1, F_a-2 & ADAM) with $f_{u,gut}$ = 1 (F_a-1) or 0.063 (F_a-2 & ADAM). [b] Predicted in vitro-to-in vivo scaling factors (SFs) for intestinal P-gp J_{max}. [c] Obs, observed; Pred, predicted; P/O, ratios of predicted to observed value. –, not calculated.

Table 4

Clinically observed and PBPK model-predicted pharmacokinetic parameters of bosutinib in bosutinib 100 mg single-dose DDI studies with ketoconazole

Group [a]	PBPK Model	J_{max} [b] SF	Ki [c] *μM*	Analysis [d]	C_{max} *ng/mL*	AUC *ng·h/mL*	$C_{max}R$ [e] *ratio*	AUCR [e] *ratio*
Control	–	–	–	Obs	7.0 (45)	323 (43)	–	–
	F_a	–	–	Pred	7.2 (45)	265 (68)	–	–
				P/O	1.03	0.82	–	–
	ADAM	–	–	Pred	38 (44)	709 (68)	–	–
				P/O	5.41	2.19	–	–
		1	–	Pred	31 (47)	653 (72)	–	–
				P/O	4.47	2.02	–	–
		25	–	Pred	8.0 (86)	276 (95)	–	–
				P/O	1.15	0.85	–	–
Test	–	–	–	Obs	38 (54)	2631 (30)	5.2 (4.3 – 6.2)	8.6 (7.5 – 9.9)
	F_a	–	–	Pred	20 (39)	1221 (88)	2.9 (2.6 – 3.2)	4.7 (3.7 – 5.7)
				P/O	0.53	0.46	0.57	0.55
	ADAM	25	–	Pred	21 (77)	1494 (85)	2.5 (2.3 – 2.9)	5.7 (5.3 – 8.1)
				P/O	0.56	0.57	0.47	0.66
		25	0.2	Pred	37 (56)	2098 (78)	5.2 (4.6 – 5.7)	11 (9.3 – 14)
				P/O	0.96	0.80	1.00	1.32

Data are expressed as mean with percent coefficient of variation (CV%) in parentheses (n = 24 for the observed; n = 6 per group × 6 groups for the predicted) except for median t_{max} with minimal to maximal values and geometric mean for $C_{max}R$ and AUCR with 90% confidence interval.
[a] Bosutinib 100 mg without and with ketoconazole 400 mg once daily (control and test groups, respectively). [b] Predicted in vitro-to-in vivo scaling factors for intestinal P-gp J_{max}. [c] Predicted ketoconazole Ki value for intestinal P-gp. [d] Obs, observed; Pred, predicted; P/O, ratios of predicted to observed value. [e] Ratios of C_{max} and AUC in test group relative to control group. –, not applicablenot or calculated.

Table 5

Clinically observed and PBPK model-predicted pharmacokinetic parameters of bosutinib in bosutinib 500 mg single-dose DDI studies with ketoconazole

Group [a]	PBPK Model	J_{max} [b] SF	Ki [c] *μM*	Analysis [d]	C_{max} *ng/mL*	AUC *ng·h/mL*	C_{max}R [e] *ratio*	AUCR [e] *ratio*
Control	–	–	–	Obs	114 (35)	2330 (35)	–	–
	F_a	–	–	Pred	84 (41)	3013 (64)	–	–
				P/O	0.74	1.29	–	–
	ADAM	–	–	Pred	153 (48)	3606 (64)	–	–
				P/O	1.34	1.55	–	–
		1	–	Pred	136 (49)	3316 (66)	–	–
				P/O	1.20	1.42	–	–
		4	–	Pred	109 (53)	2738 (71)	–	–
				P/O	0.95	1.18	–	–
Test	–	–	–	Obs	326 (24)	15200 (29)	2.9	6.5
	F_a	–	–	Pred	228 (32)	13311 (69)	2.8 (2.5 – 3.1)	4.2 (3.3 – 5.2)
				P/O	0.70	0.88	0.97	0.66
	ADAM	4	–	Pred	226 (43)	10639 (61)	2.1 (2.0 – 2.3)	4.0 (3.1 – 4.9)
				P/O	0.69	0.70	0.72	0.60
		4	0.2	Pred	272 (40)	12127 (57)	2.6 (2.4 – 2.8)	4.7 (3.7 – 5.7)
				P/O	0.83	0.80	0.87	0.70

Data are expressed as mean with percent coefficient of variation (CV%) in parentheses (n = 54-56 for the observed; n = 6 per group × 6 groups for the predicted) except for median t_{max} with minimal to maximal values and geometric mean for C_{max}R and AUCR with 90% confidence interval.

[a] Bosutinib 500 mg without and with ketoconazole 400 mg once daily (control and test groups, respectively). [b] Predicted in vitro-to-in vivo scaling factors for intestinal P-gp J_{max}. [c] Predicted ketoconazole Ki value for intestinal P-gp. [d] Obs, observed; Pred, predicted; P/O, ratios of predicted to observed value. [e] Ratios of C_{max} and AUC in test group relative to control group. –, not applicablenot or calculated.

Table 6

Clinically observed and PBPK model-predicted pharmacokinetic parameters of bosutinib in bosutinib 500 mg single-dose DDI studies with rifampin

Group [a]	PBPK Model	J_{max} [b] SF	Induction [c] fold	Analysis [d]	C_{max} *ng/mL*	AUC *ng·h/mL*	$C_{max}R$ [e] *ratio*	AUCR [e] *ratio*
Control	–	–	–	Obs	112 (26)	2740 (29)	–	–
	F_a	–	–	Pred	83 (40)	3005 (65)	–	–
				P/O	0.74	1.10	–	–
	ADAM	–	–	Pred	153 (48)	3606 (64)	–	–
				P/O	1.37	1.32	–	–
		1	–	Pred	136 (49)	3316 (66)	–	–
				P/O	1.22	1.21	–	–
		4	–	Pred	109 (53)	2738 (71)	–	–
				P/O	0.97	1.00	–	–
Test	–	–	–	Obs	16 (42)	207 (22)	0.14 (0.12 – 0.16)	0.08 (0.07 – 0.09)
	F_a	–	–	Pred	26 (71)	487 (76)	0.27 (0.23 – 0.31)	0.14 (0.12 –0.17)
				P/O	1.62	2.35	1.92	1.86
	ADAM	4	–	Pred	41 (64)	628 (89)	0.34 (0.30 – 0.38)	0.21 (0.18 – 0.24)
				P/O	2.56	3.03	2.46	2.84
		4	10	Pred	16 (64)	260 (106)	0.13 (0.11 – 0.15)	0.08 (0.06 – 0.09)
				P/O	1.01	1.26	0.86	0.95

Data are expressed as mean with percent coefficient of variation (CV%) in parentheses (n = 22-24 for the observed; n = 6 per group × 6 groups for the predicted) except for median t_{max} with minimal to maximal values and geometric mean for $C_{max}R$ and AUCR with 90% confidence interval.

[a] Bosutinib 500 mg without and with rifampin 600 mg once daily (control and test groups, respectively). [b] Predicted in vitro-to-in vivo scaling factors for intestinal P-gp J_{max}. [c] Predicted rifampin-mediated fold increase in intestinal P-gp. [d] Obs, observed; Pred, predicted; P/O, ratios of predicted to observed value. [e] Ratios of C_{max} and AUC in test group relative to control group. –, not applicablenot or calculated.

Table 7

PBPK-ADAM model-predicted pharmacokinetic parameters of bosutinib in bosutinib 500 mg single-dose DDI studies with verapamil and itraconazole

PBPK Model	Precipitant drug	Group [a]	Ki [b] *μM*	C_{max} *ng/mL*	AUC *ng·h/mL*	$C_{max}R$ [c] *ratio*	AUCR [c] *ratio*
ADAM	Itraconazole	Control	–	114 (51)	2578 (71)	–	–
		Test	–	228 (46)	21108 (123)	2.0 (1.9 – 2.1)	8.5 (6.5 – 10)
			0.5	229 (46)	21159 (123)	2.0 (1.9 – 2.1)	8.5 (6.5 – 10)
	Verapamil	Control	–	112 (49)	2528 (70)	–	–
		Test	–	190 (50)	11269 (110)	1.7 (1.6 – 1.8)	4.5 (3.7 – 5.3)
			0.16	226 (49)	13015 (111)	2.0 (1.9 – 2.2)	5.1 (4.3 – 6.2)

Data are expressed as geometric mean with percent coefficient of variation (CV%) except for $C_{max}R$ and AUCR with 90% confidence interval in parentheses (n = 6 per group × 6 groups for the predicted)

[a] Bosutinib 500 mg without and with itraconazole 200 mg once daily (control and test groups, respectively) or verapamil 80 mg three times a day. [b] Ki for intestinal P-gp. [c] Ratios of C_{max} and AUC in test group relative to control group. –, not applicable.

Figure 1

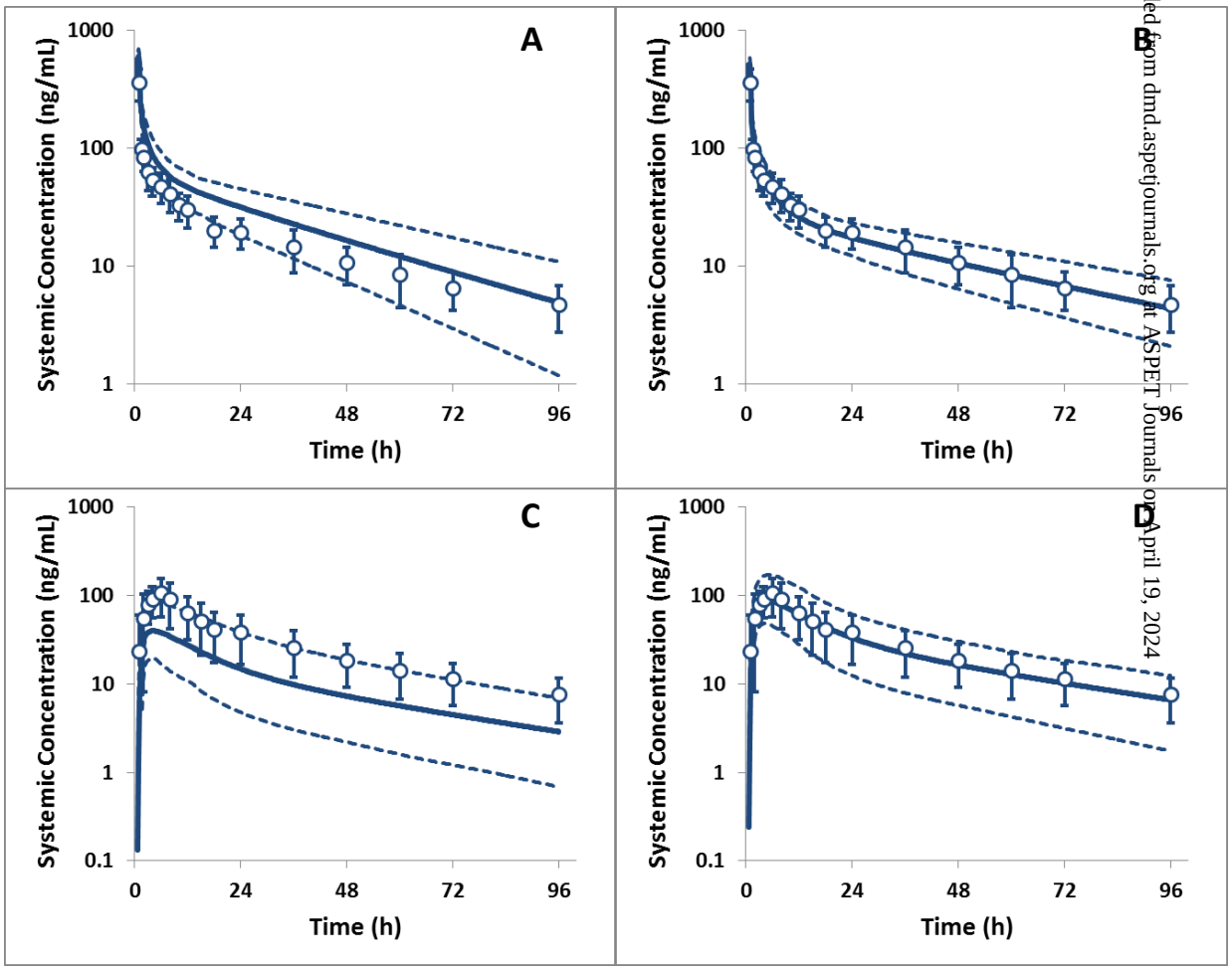

Figure 2

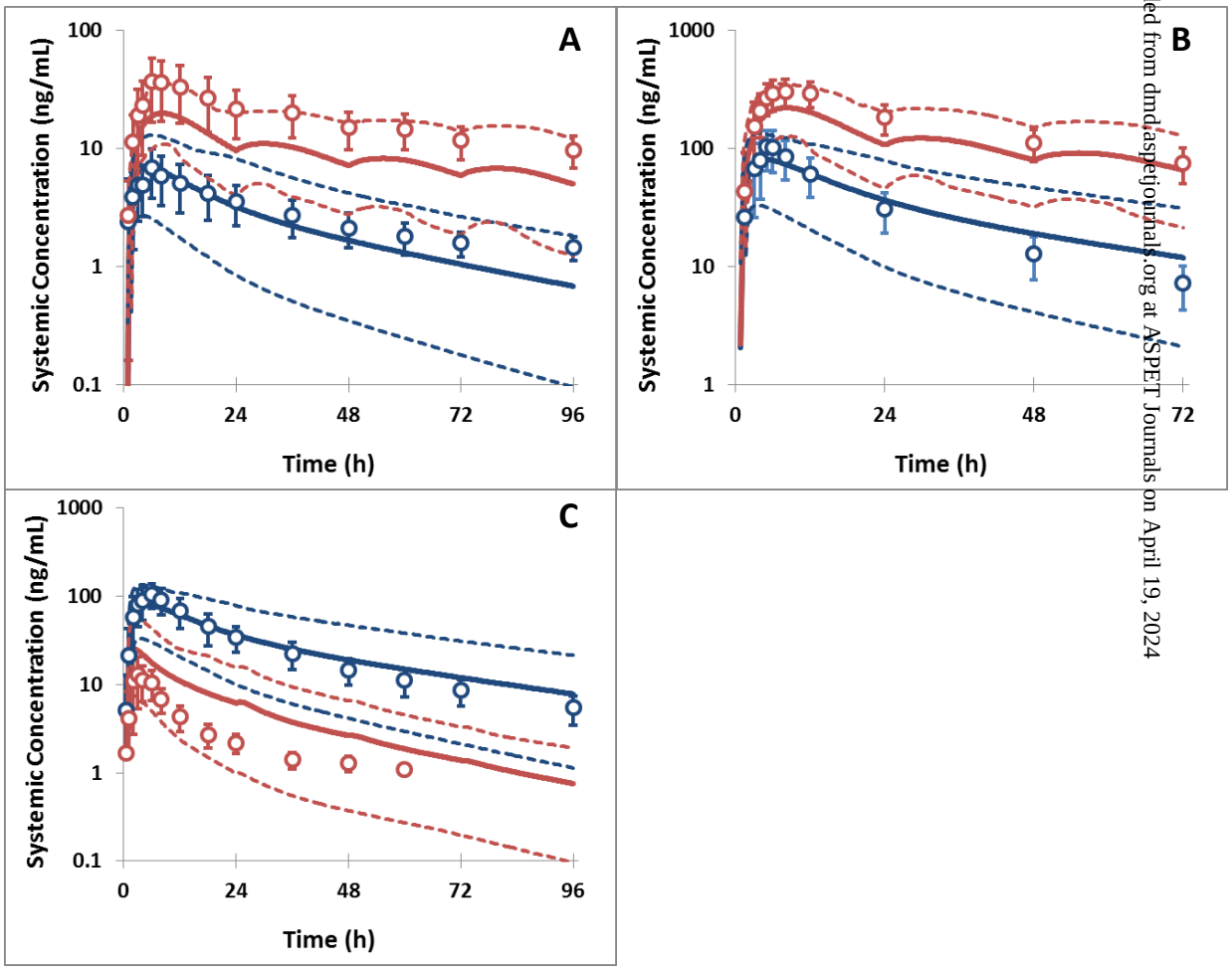

Figure 3

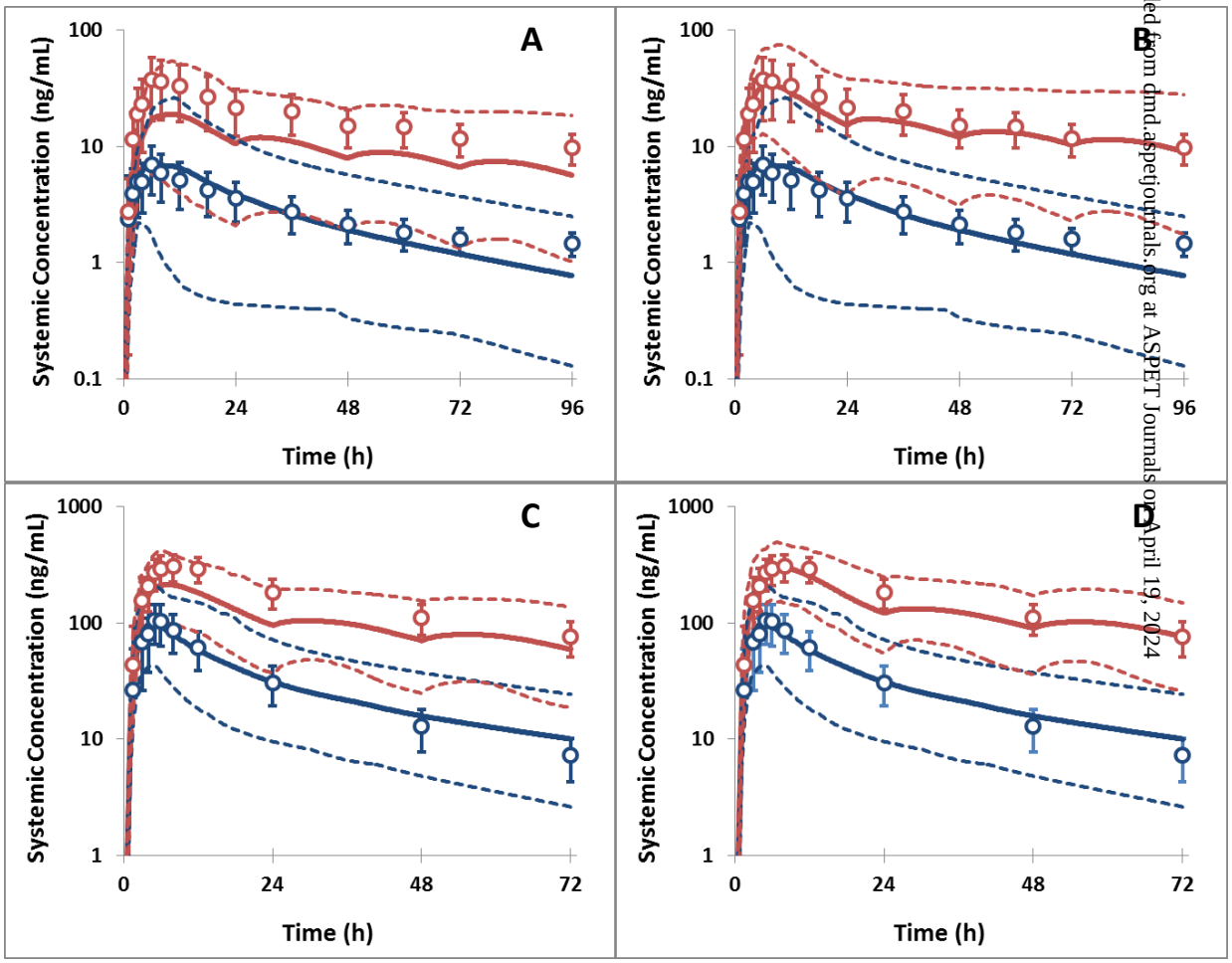

Figure 4